```
   1 ATGGCGCCAC CACCAGCTAG AGTACATCTA GGTGCGTTCC TGGCAGTGAC TCGCAATGCG GCGAGCGCAG CGAGTGGGAC AGAGGCAGCC GCGGCCACAC
     TACCGCGGTG GTGGTCGATC TCATGTAGAT CCACGCAAGG ACCGTCACTG AGGCTTAGGG CCCTCGCGTC GCTCACCCTG TCTCCGTCGG CGCCGGTGTG
   1 MetAlaProP roProAlaAr gValHisLeu GlyAlaPheL euAlaValTh rProAsnPro GlySerAlaA laSerGlyTh rGluAlaAla AlaAlaThrPro

 101 CCAGCAAAGT GTGGGGCTCT TCCGCGGGGA GGATTGAACC ACGAGGCGGG GGCCGAGGAG CGCTCCCTAC CTCCATGGGA CAGCACGGAC CCAGTGCCCG
     GGTCGTTTCA CACCCCGAGA AGGCGCCCCT CCTAACTTGG TGCTCCGCCC CCGGCTCCTC GCGAGGGATG GAGGTACCCT GTCGTGCCTG GGTCACGGGC
  35   SerLysVa lTrpGlySer SerAlaGlyA rgIleGluPr oArgGlyGly GlyArgGlyA laLeuProTh rSerMetGly GlnHisGlyP roSerAlaArg

 201 GGCCCGGGCA GGGCGCGCCC CAGGACCCAG GCCGGCGCGG GAAGCCAGCC CTCGGCTCCG GGTCCACAAG ACCTTCAAGT TTGTCGTCGT CGGGGTCCTG
     CCGGGCCCGT CCCGCGCGGG GTCCTGGGTC CGGCCGCGCC CTTCGGTCGG GAGCCGAGGC CCAGGTGTTC TGGAAGTTCA AACAGCAGCA GCCCCAGGAC
  68  AlaArgAla GlyArgAlaP roGlyProAr gProAlaArg GluAlaSerP roArgLeuAr gValHisLys ThrPheLysP heValValVa lGlyValLeu

 301 CTGCAGGTCG TACCTAGCTC AGCTGCAACC ATCAAACTTC ATGATCAATC AATTGGCACA CAGCAATGGG AACATAGCCC TTTGGGAGAG TTGTGTCCAC
     GACGTCCAGC ATGGATCGAG TCGACGTTGG TAGTTTGAAG TACTAGTTAG TTAACCGTGT GTCGTTACCC TTGTATCGGG AAACCCTCTC AACACAGGTG
 101 LeuGlnValV alProSerSe rAlaAlaThr IleLysLeuH isAspGlnSe rIleGlyThr GlnGlnTrpG luHisSerPr oLeuGlyGlu LeuCysProPr

 401 CAGGATCTCA TAGATCAGAA CGTCCTGGAG CCTGTAACCG GTGCACAGAG GGTGTGGGTT ACACCAATGC TTCCAACAAT TTGTTTGCTT GCCTCCCATG
     GTCCTAGAGT ATCTAGTCTT GCAGGACCTC GGACATTGGC CACGTGTCTC CCACACCCAA TGTGGTTACG AAGGTTGTTA AACAAACGAA CGGAGGGTAC
 135   GlySerHi sArgSerGlu ArgProGlyA laCysAsnAr gCysThrGlu GlyValGlyT yrThrAsnAl aSerAsnAsn LeuPheAlaC ysLeuProCys

 501 TACAGCTTGT AAATCAGATG AAGAAGAGAG AAGTCCCTGC ACCACGACCA GGAACACAGC ATGTCAGTGC AAACCAGGAA CTTTCCGGAA TGACAATTCT
     ATGTCGAACA TTTAGTCTAC TTCTTCTCTC TTCAGGGACG TGGTGCTGGT CCTTGTGTCG TACAGTCACG TTTGGTCCTT GAAAGGCCTT ACTGTTAAGA
 168  ThrAlaCys LysSerAspG luGluGluAr gSerProCys ThrThrThrA rgAsnThrAl aCysGlnCys LysProGlyT hrPheArgAs nAspAsnSer

 601 GCTGAGATGT GCCGGAAGTG CAGCACAGGG TGCCCCAGAG GGATGGTCAA GGTCAAGGAT TGTACGCCCT GGAGTGACAT CGAGTGTGTC CACAAAGAAT
     CGACTCTACA CGGCCTTCAC GTCGTGTCCC ACGGGGTCTC CCTACCAGTT CCAGTTCCTA ACATGCGGGA CCTCACTGTA GCTCACACAG GTGTTTCTTA
 201 AlaGluMetC ysArgLysCy sSerThrGly CysProArgG lyMetValLy sValLysAsp CysThrProT rpSerAspIl eGluCysVal HisLysGluSer

 701 CAGGCAATGG ACATAATATA TGGGTGATTT TGGTTGTGAC TTTGGTTGTT CCGTTGCTGT TGGTGGCTGT GCTGATTGTC TGTTGTTGCA TCGGCTCAGG
     GTCCGTTACC TGTATTATAT ACCCACTAAA ACCAACACTG AAACCAACAA GGCAACGACA ACCACCGACA CGACTAACAG ACAACAACGT AGCCGAGTCC
 235   GlyAsnGl yHisAsnIle TrpValIleL euValValTh rLeuValVal ProLeuLeuL euValAlaVa lLeuIleVal CysCysCysI leGlySerGly

 801 TTGTGGAGGG GACCCCAAGT GCATGGACAG GGTGTGTTTC TGGCGCTTGG GTCTCCTACG AGGGCCTGGG GCTGAGGACA ATGCTCACAA CGAGATTCTG
     AACACCTCCC CTGGGGTTCA CGTACCTGTC CCACACAAAG ACCGCGAACC CAGAGGATGC TCCCGGACCC CGACTCCTGT TACGAGTGTT GCTCTAAGAC
 268  CysGlyGly AspProLysC ysMetAspAr gValCysPhe TrpArgLeuG lyLeuLeuAr gGlyProGly AlaGluAspA snAlaHisAs nGluIleLeu

 901 AGCAACGCAG ACTCGCTGTC CACTTTCGTC TCTGAGCAGC AAATGGAAAG CCAGGAGCCG GCAGATTTGA CAGGTGTCAC TGTACAGTCC CCAGGGGAGG
     TCGTTGCGTC TGAGCGACAG GTGAAAGCAG AGACTCGTCG TTTACCTTTC GGTCCTCGGC CGTCTAAACT GTCCACAGTG ACATGTCAGG GGTCCCCTCC
 301 SerAsnAlaA spSerLeuSe rThrPheVal SerGluGlnG lnMetGluSe rGlnGluPro AlaAspLeuT hrGlyValTh rValGlnSer ProGlyGluAla

1001 CACAGTGTCT GCTGGGACCG GCAGAAGCTG AAGGGTCTCA GAGGAGGAGG CTGCTGGTTC CAGCAAATGG TGCTGACCCC ACTGAGACTC TGATGCTGTT
     GTGTCACAGA CGACCCTGGC CGTCTTCGAC TTCCCAGAGT CTCCTCCTCC GACGACCAAG GTCGTTTACC ACGACTGGGG TGACTCTGAG ACTACGACAA
 335   GlnCysLe uLeuGlyPro AlaGluAlaG luGlySerGl nArgArgArg LeuLeuValP roAlaAsnGl yAlaAspPro ThrGluThrL euMetLeuPhe
```

Fig. 1

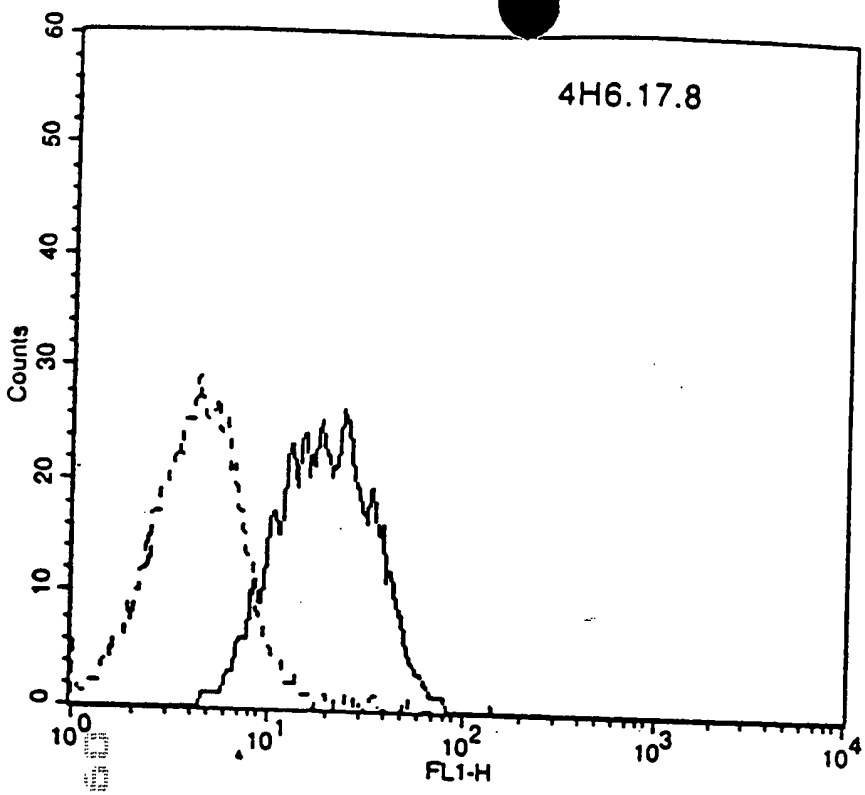
```
1101 CTTTGACAAG TTTGCAAACA TCGTGCCCTT TGACTCCTGG GACCAGCTCA TCGTGGACCT GGACCTCACG AAAAATGAGA TCGATGTGGT CAGAGCTGGT
     GAAACTGTTC AAACGTTTGT AGCACGGGAA ACTGAGGACC CTGGTCGAGT ACTCCGTCGA CCTGGAGTGC TTTTTACTCT AGCTACACCA GTCTCGACCA
 368  PheAspLys PheAlaAsnI leValProPh eAspSerTrp AspGlnLeuM etArgGlnLe uAspLeuThr LysAsnGluI leAspValVa lArgAlaGly

1201 ACAGCAGGCC CAGGGGATGC CTTGTATGCA ATGCTGATGA AATGGGTCAA CAAAACTGGA CGGAACGCCT CGATCCACAC CCTGCTGGAT GCCTTGGAGA
     TGTCGTCCGG GTCCCCTACG GAACATACGT TACGACTACT TTACCCAGTT GTTTTGACCT GCCTTGCGGA GCTAGGTGTG GGACGACCTA CGGAACCTCT
 401 ThrAlaGlyP roGlyAspAl aLeuTyrAla MetLeuMetL ysTrpValAs nLysThrGly ArgAsnAlaS erIleHisTh rLeuLeuAsp AlaLeuGluArg

1301 GGATGGAAGA GAGACATGCA AAAGAGAAGA TTCAGGACCT CTTGGTGGAC TCTGGAAAGT TCATCTACTT AGAAGATGGC ACAGGCTCTG CCGTGTCCTT
     CCTACCTTCT CTCTGTACGT TTTCTCTTCT AAGTCCTGGA GAACCACCTG AGACCTTTCA AGTAGATGAA TCTTCTACCG TGTCCGAGAC GGCACAGGAA
 435   MetGluGl uArgHisAla LysGluLysI leGlnAspLe uLeuValAsp SerGlyLysP heIleTyrLe uGluAspGly ThrGlySerA laValSerLeu

1401 GGAGTGA
     CCTCACT
 468  GluOP*
```

Fig. 1 (cont.)


4H6.17.8
Counts
0
10
20
30
40
50
60
10^0
10^1
10^2
10^3
10^4
FL1-H

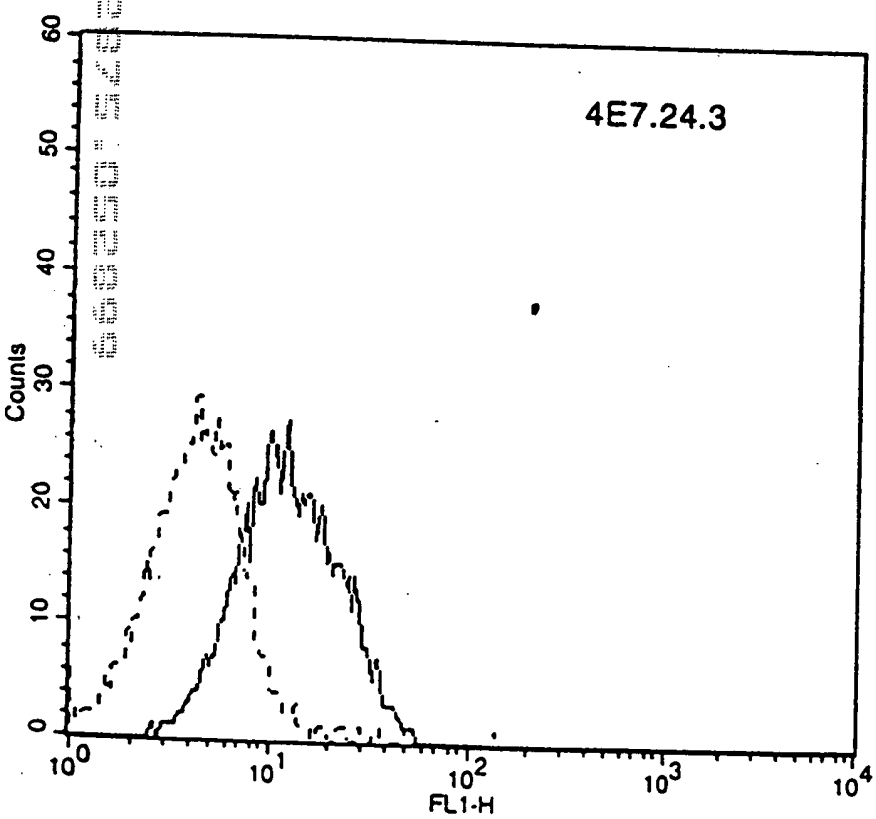

4E7.24.3
Counts
0
10
20
30
40
50
60
10^0
10^1
10^2
10^3
10^4
FL1-H

Fig. 2

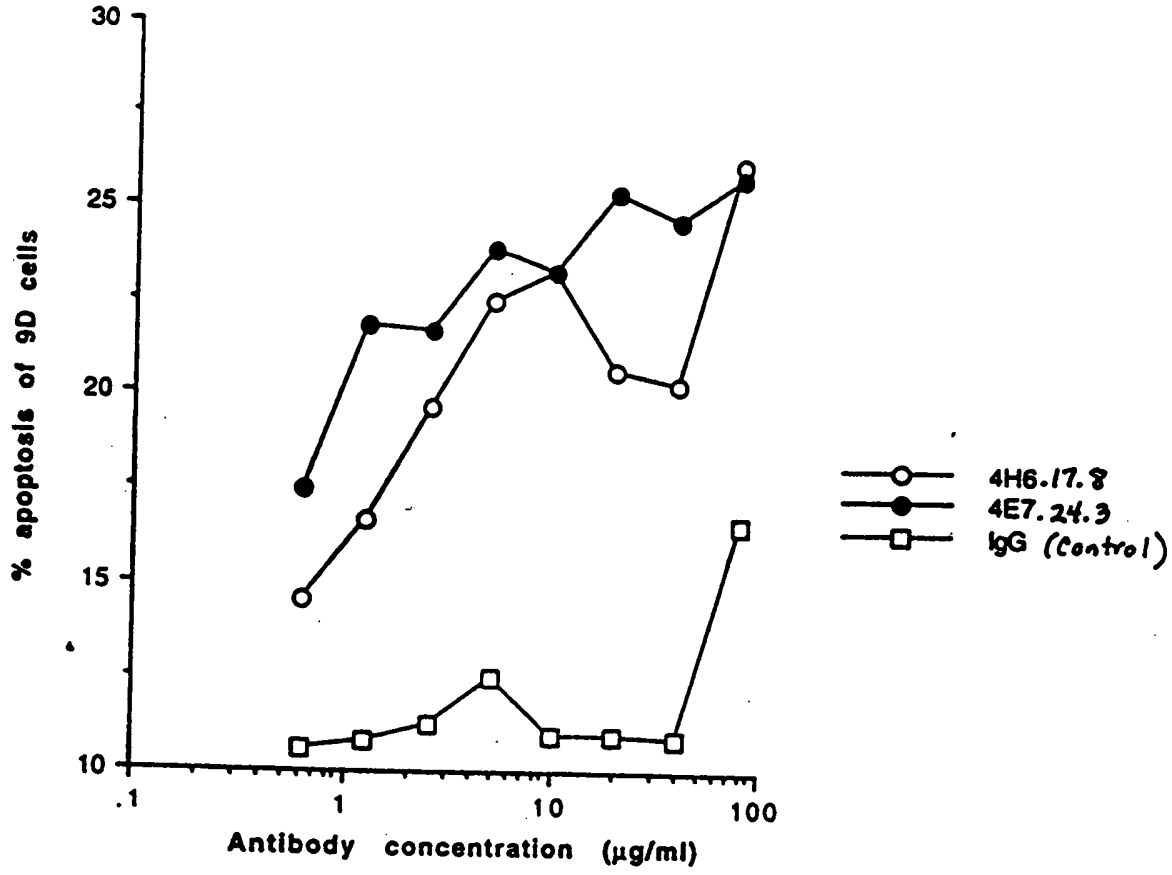

30
25
20
15
10
% apoptosis of 9D cells
.1
1
10
100
Antibody concentration (µg/ml)
4H6.17.8
4E7.24.3
IgG (Control)

Fig. 3

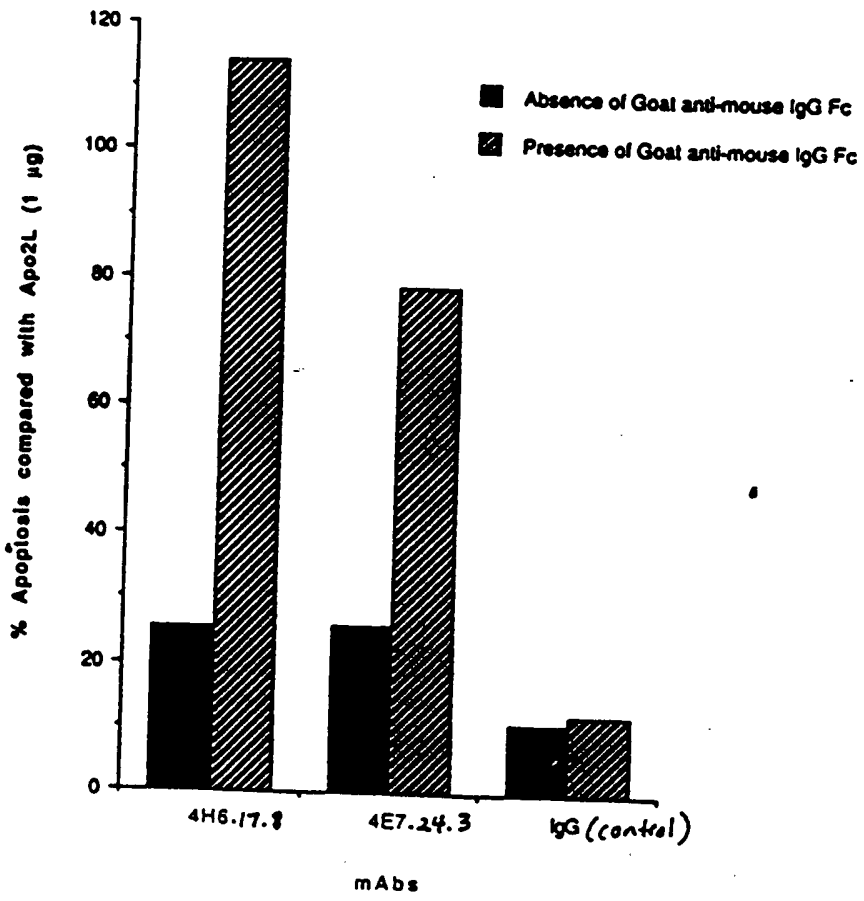

120
100
80
60
40
20
0
% Apoptosis compared with Apo2L (1 µg)
Absence of Goat anti-mouse IgG Fc
Presence of Goat anti-mouse IgG Fc
4H6.17.8
4E7.24.3
IgG (control)
mAbs

Fig. 4

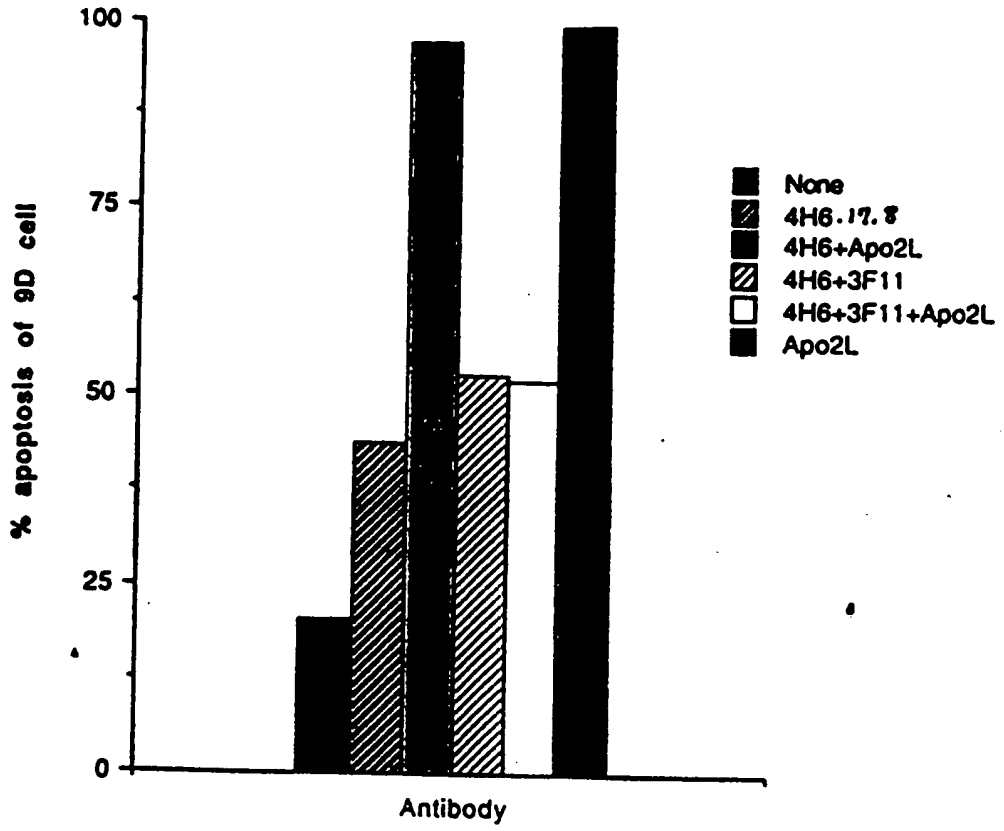
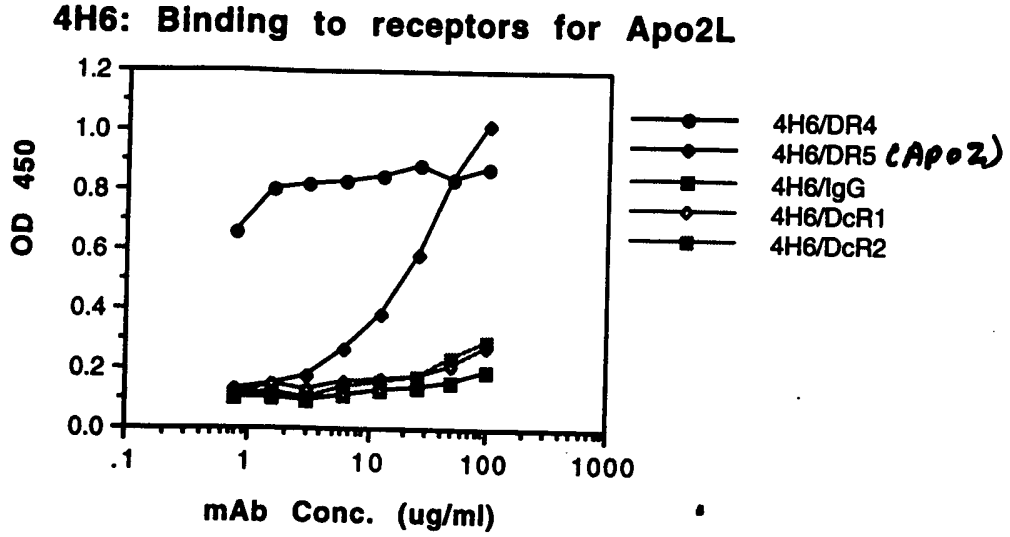

100
75
50
25
0
% apoptosis of 9D cell
Antibody
None
4H6.17.8
4H6+Apo2L
4H6+3F11
4H6+3F11+Apo2L
Apo2L

Fig. 5


4H6: Binding to receptors for Apo2L
OD 450
1.2
1.0
0.8
0.6
0.4
0.2
0.0
.1
1
10
100
1000
mAb Conc. (ug/ml)
4H6/DR4
4H6/DR5 (Apo2)
4H6/IgG
4H6/DcR1
4H6/DcR2

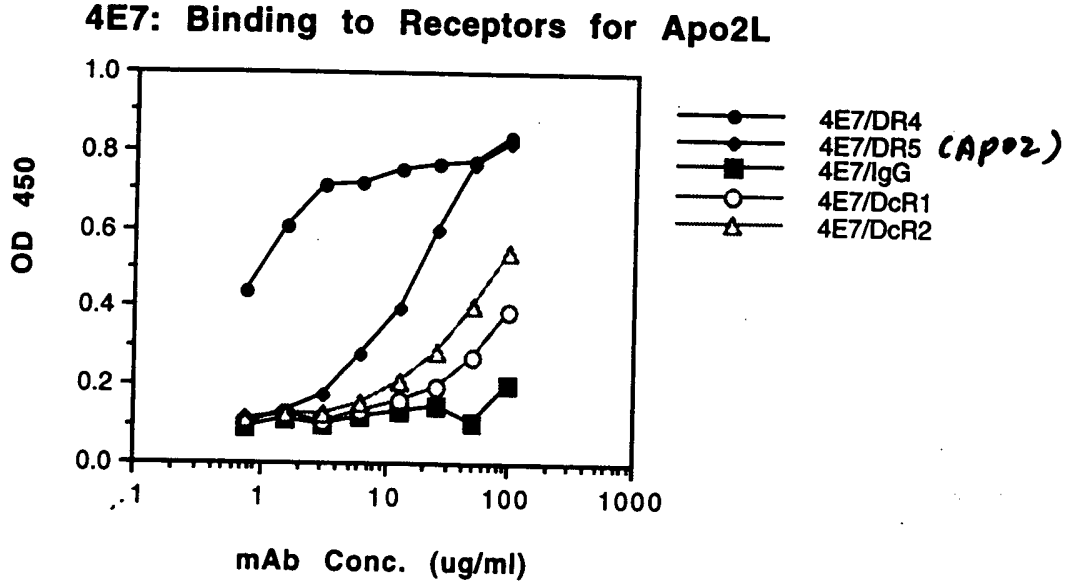

4E7: Binding to Receptors for Apo2L
OD 450
1.0
0.8
0.6
0.4
0.2
0.0
.1
1
10
100
1000
mAb Conc. (ug/ml)
4E7/DR4
4E7/DR5 (Apo2)
4E7/IgG
4E7/DcR1
4E7/DcR2

Fig. 6

Affinities of Apo2Rs and mAbs

		Affinity (pM)
DR4-IgG	to Apo2L	82
DR5-IgG	to Apo2L	1
mAb 4E7	to DR4-IgG	2
mAb 4H6	to "	5
mAb 5G11	to "	22
mAb 3F11	to DR5-IgG	20
mAb 3H3	to "	3

Affinities were determined using KinExA

Fig. 7

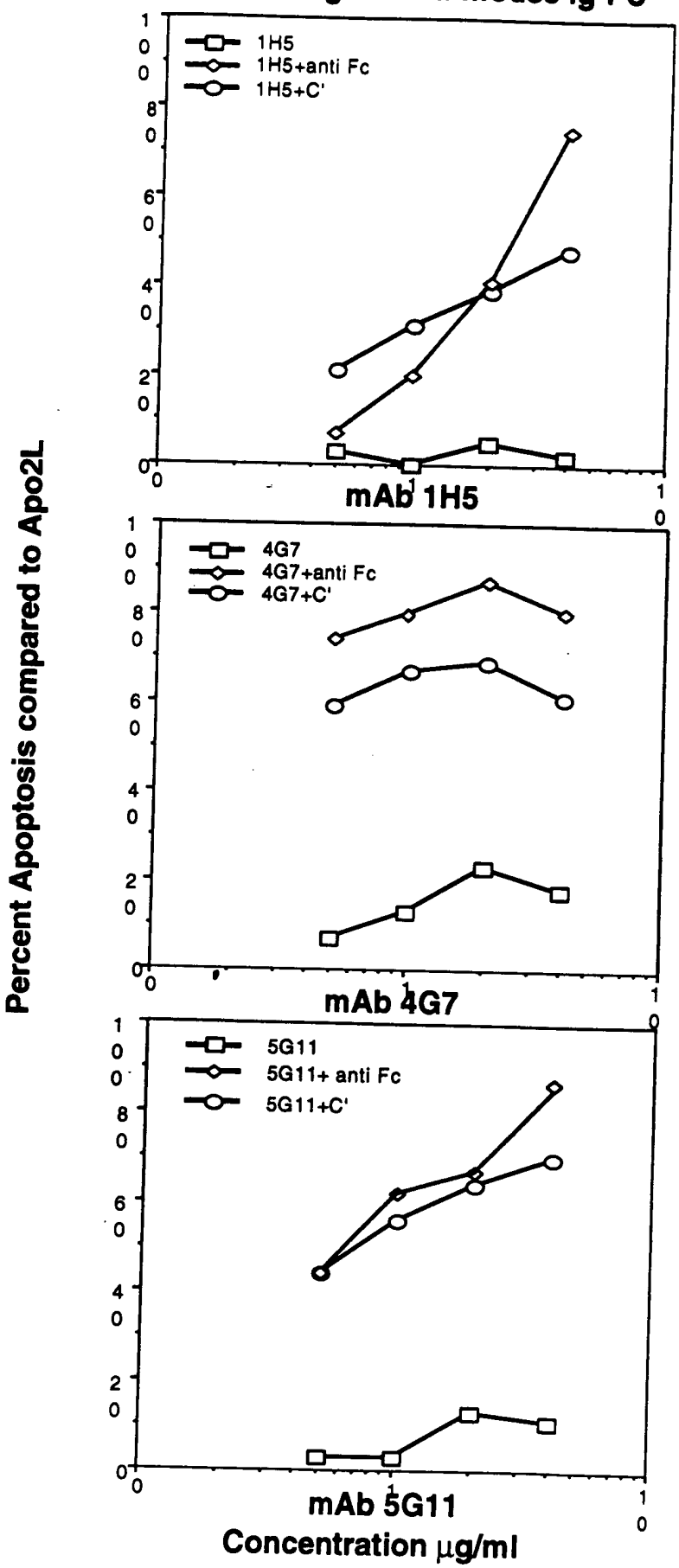
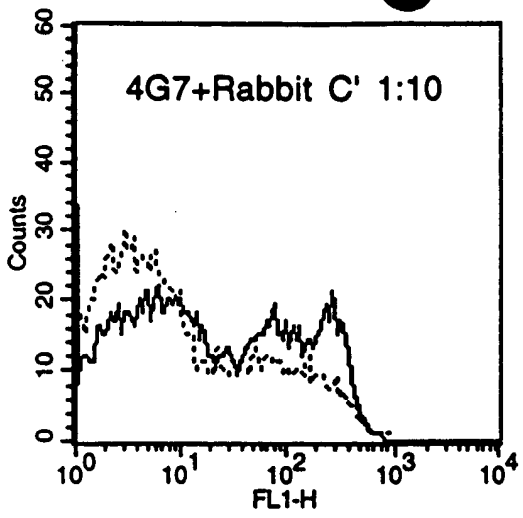
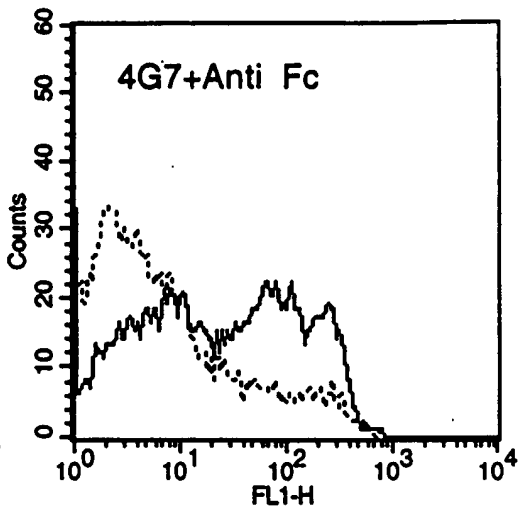
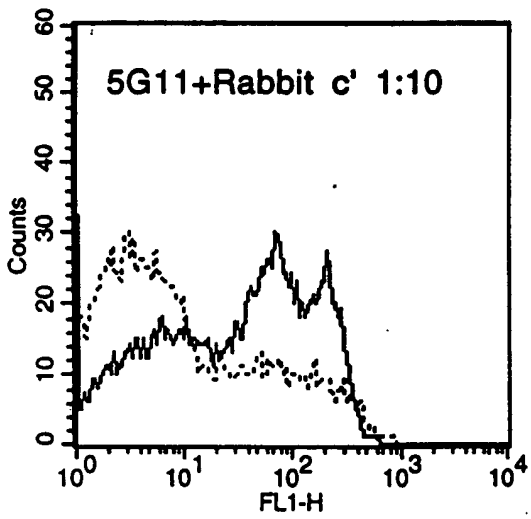
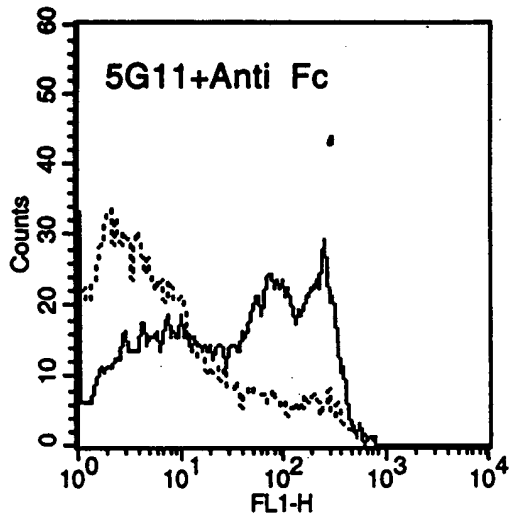

Apoptosis of 9D cells by anti-DR4 mAb plus complements or goat anti-mouse Ig-FC
1H5
1H5+anti Fc
1H5+C'
mAb 1H5
4G7
4G7+anti Fc
4G7+C'
mAb 4G7
5G11
5G11+ anti Fc
5G11+C'
mAb 5G11
Percent Apoptosis compared to Apo2L
Concentration μg/ml

Fig. 8A


4G7+Rabbit C' 1:10
Counts
FL1-H


4G7+Anti Fc
Counts
FL1-H


5G11+Rabbit c' 1:10
Counts
FL1-H


5G11+Anti Fc
Counts
FL1-H

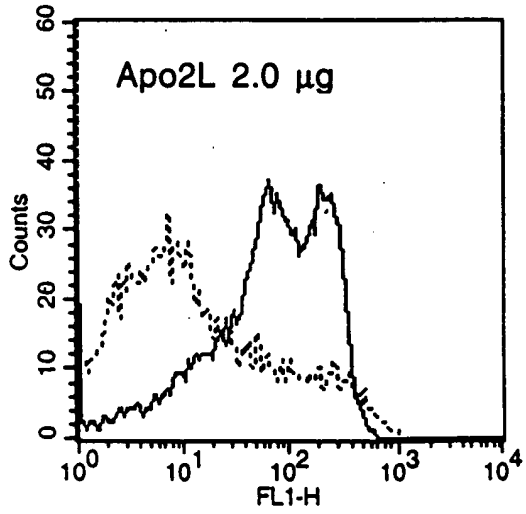

Apo2L 2.0 µg
Counts
FL1-H

Fig. 8B

Fig. 9

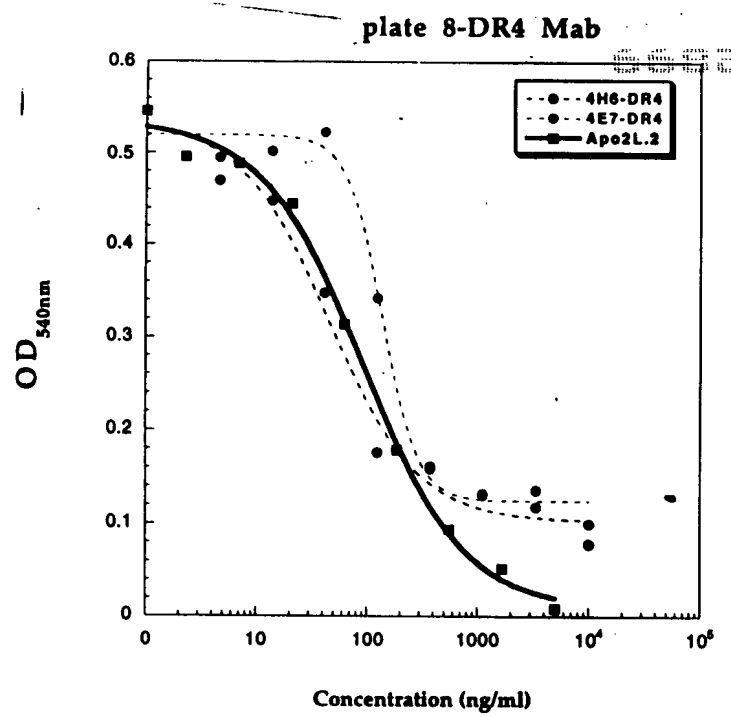


plate 8-DR4 Mab
4H6-DR4
4E7-DR4
Apo2L.2
OD540nm
Concentration (ng/ml)

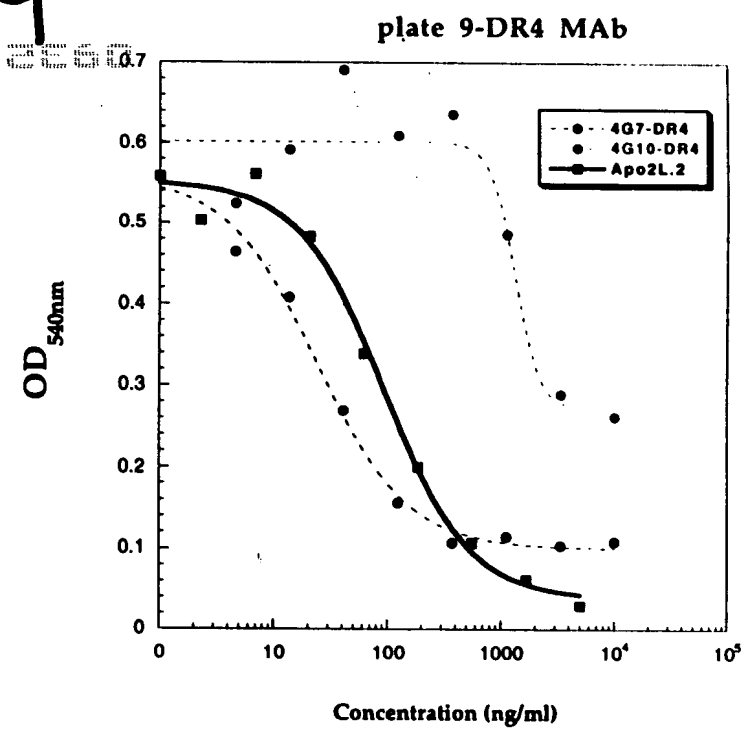


plate 9-DR4 MAb
4G7-DR4
4G10-DR4
Apo2L.2
OD540nm
Concentration (ng/ml)

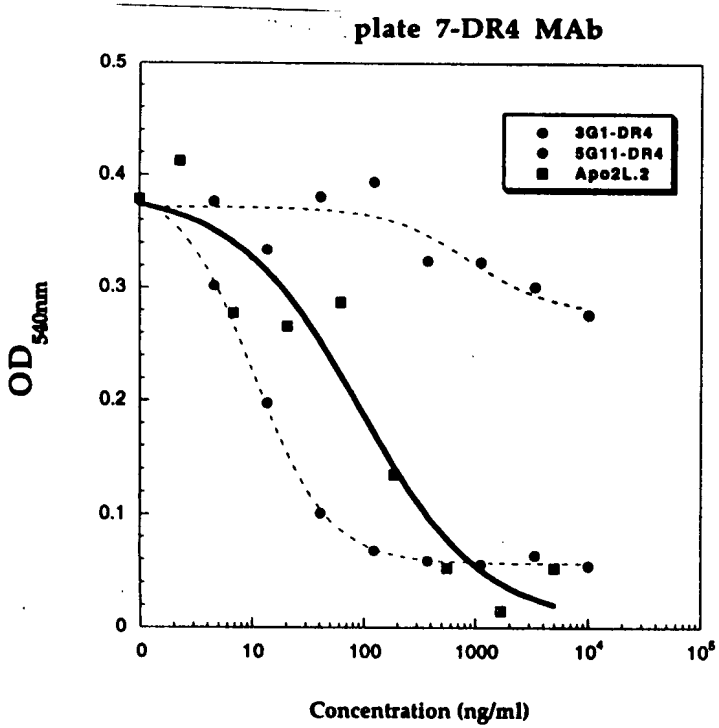


plate 7-DR4 MAb
3G1-DR4
5G11-DR4
Apo2L.2
OD540nm
Concentration (ng/ml)

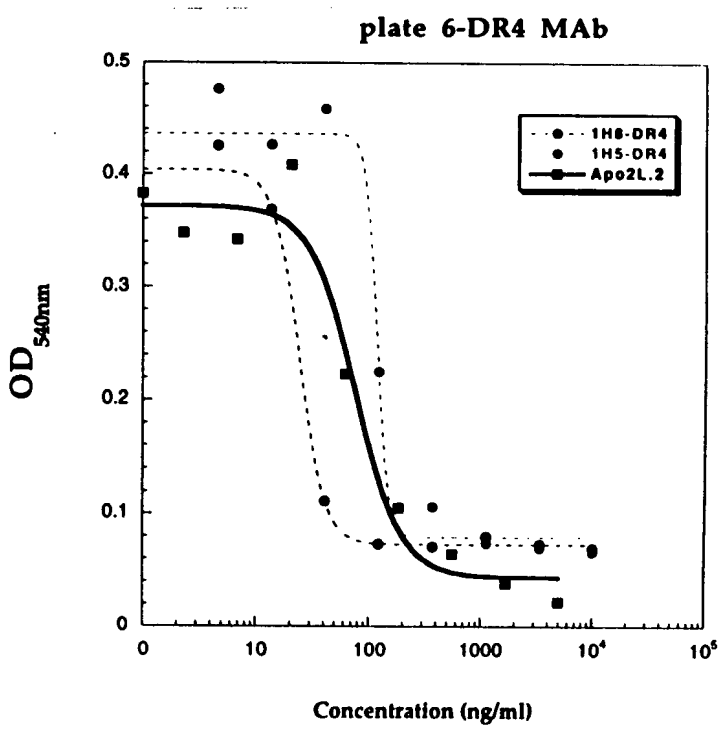
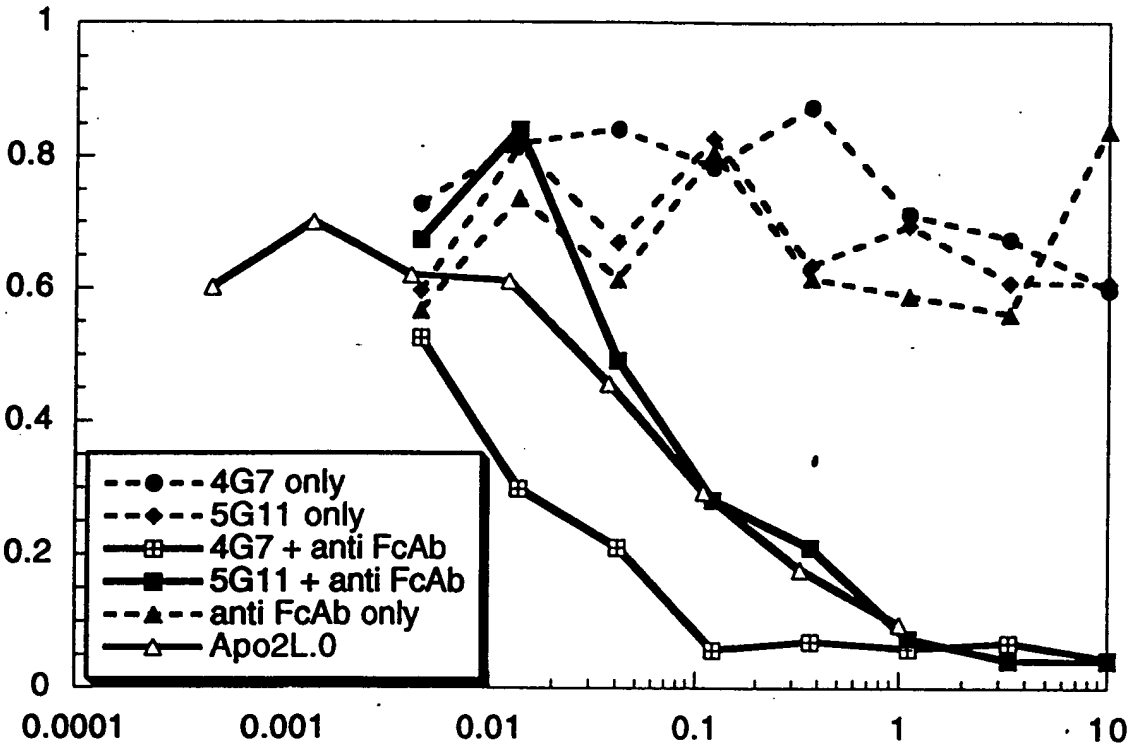


plate 6-DR4 MAb
1H6-DR4
1H5-DR4
Apo2L.2
OD540nm
Concentration (ng/ml)

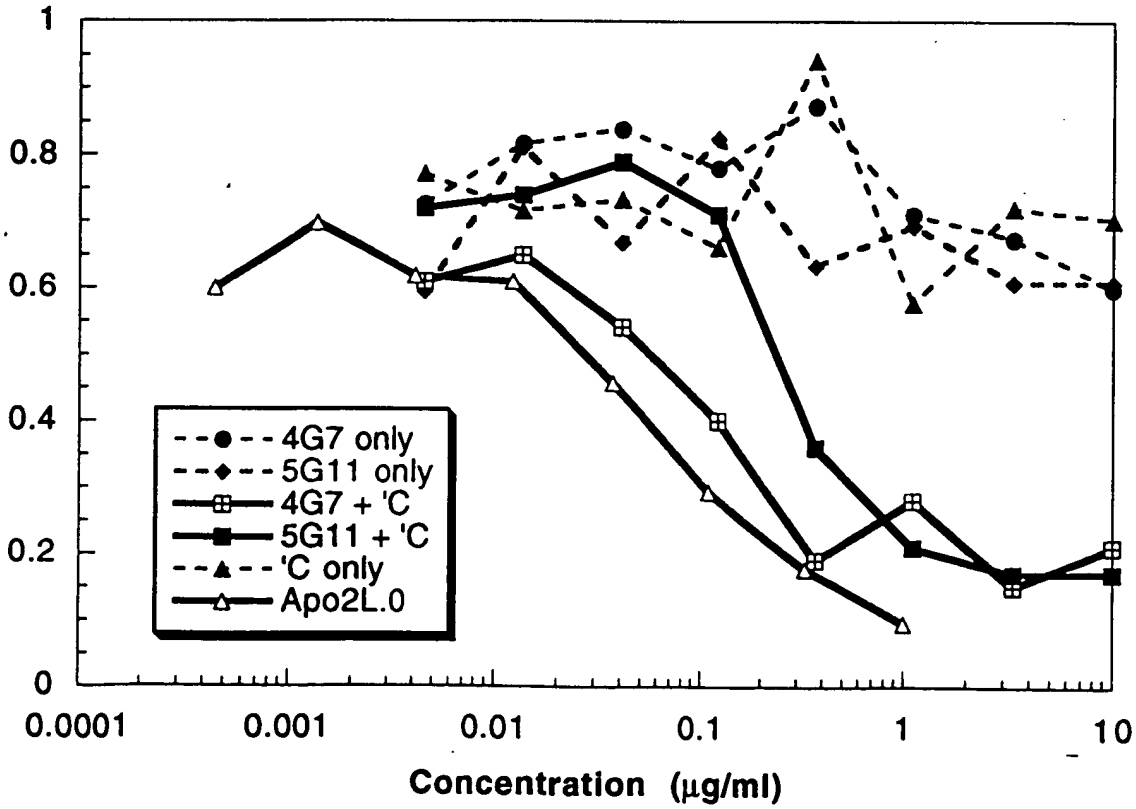
Apoptosis of anti DR4 mAbs plus goat anti FcAb



O D_{540}

Fig.10A

Apoptosis induced by anti DR4 mAbs plus Complements



OD 540

Fig.10B

Effect of anti DR4 MAb with complement on HCT 116

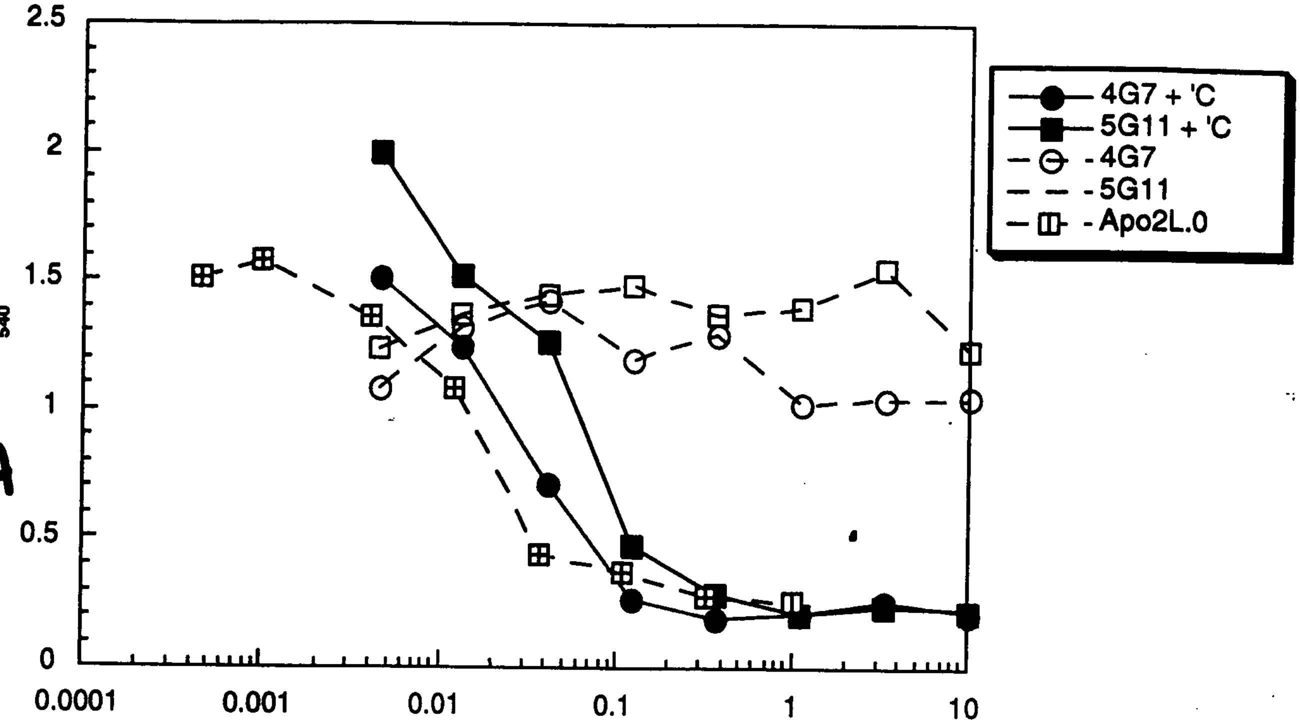


Fig. 11A

Effect of anti DR4 MAb with anti-IgFc on HCT 116

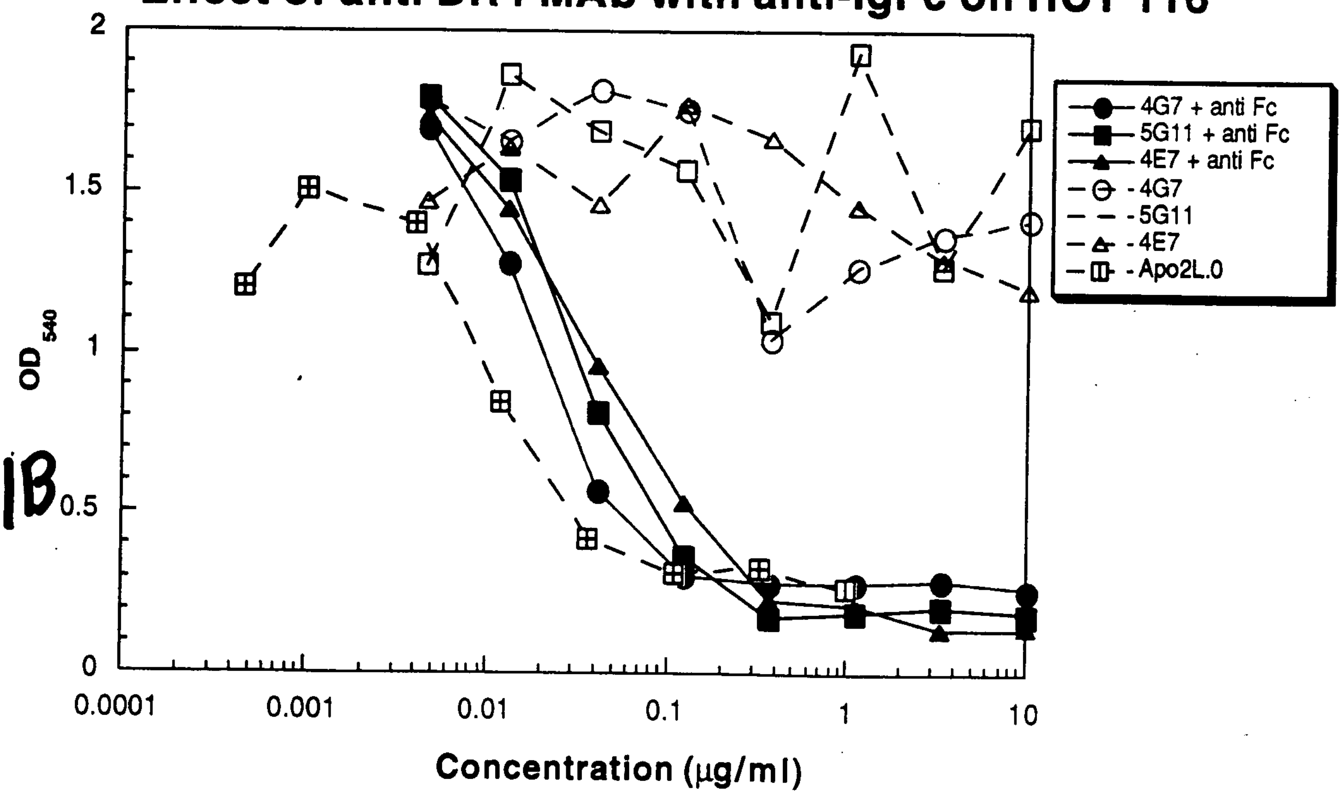


Fig. 11B

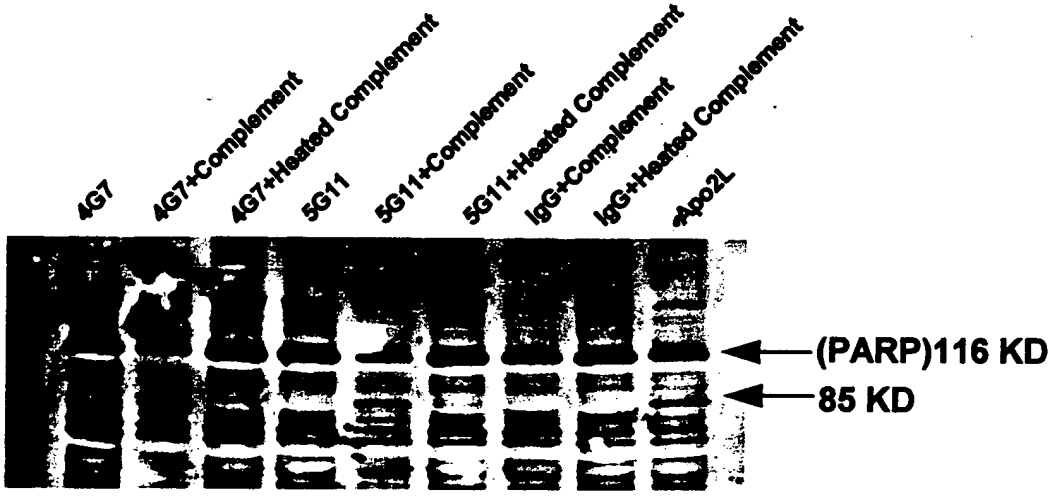

4G7
4G7+Complement
4G7+Heated Complement
5G11
5G11+Complement
5G11+Heated Complement
IgG+Complement
IgG+Heated Complement
Apo2L
(PARP)116 KD
85 KD

Fig. 12

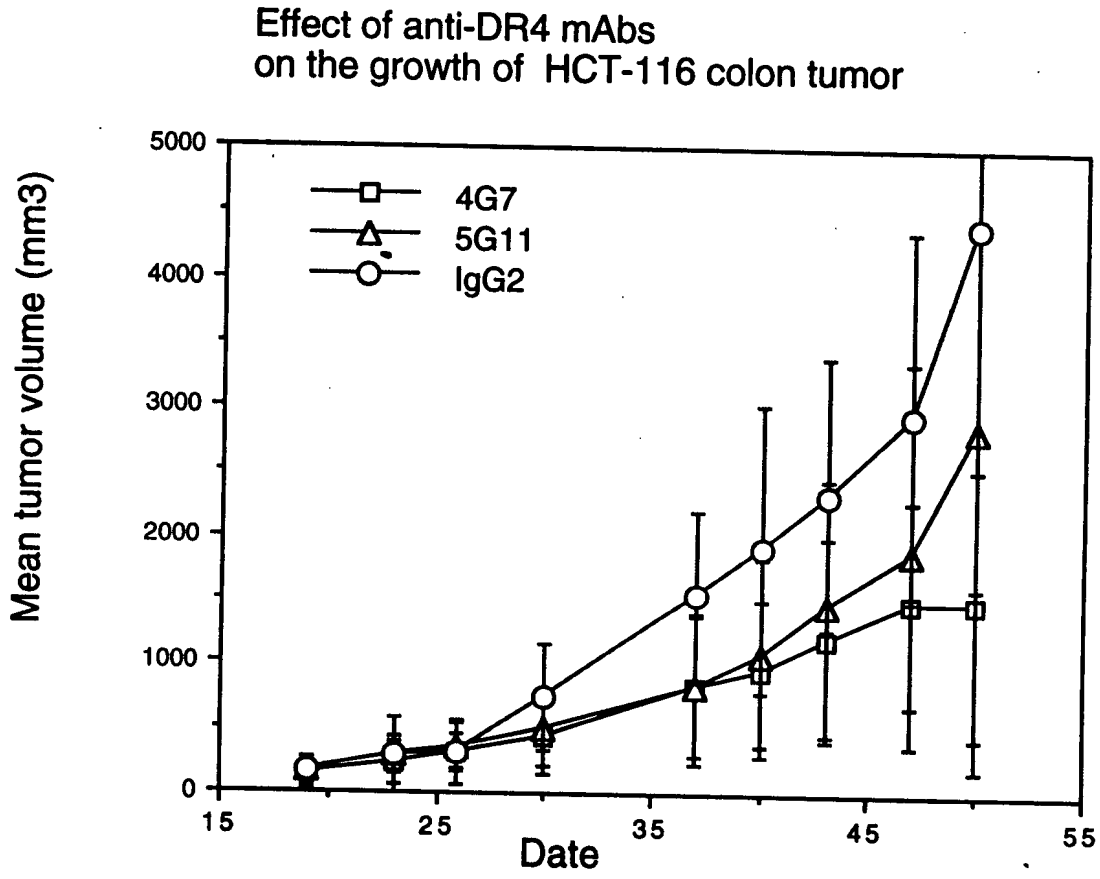

Effect of anti-DR4 mAbs
on the growth of HCT-116 colon tumor
Mean tumor volume (mm3)
5000
4000
3000
2000
1000
0
4G7
5G11
IgG2
15
25
35
45
55
Date

Fig. 13

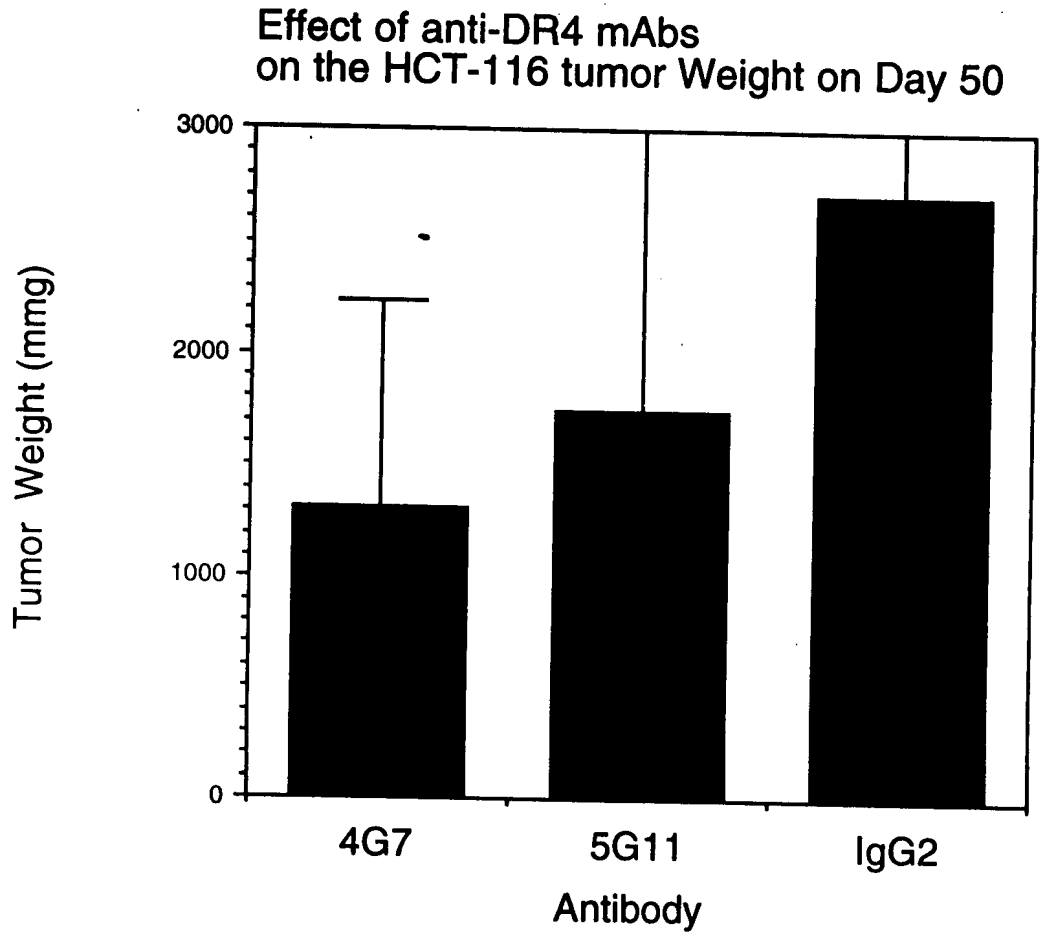
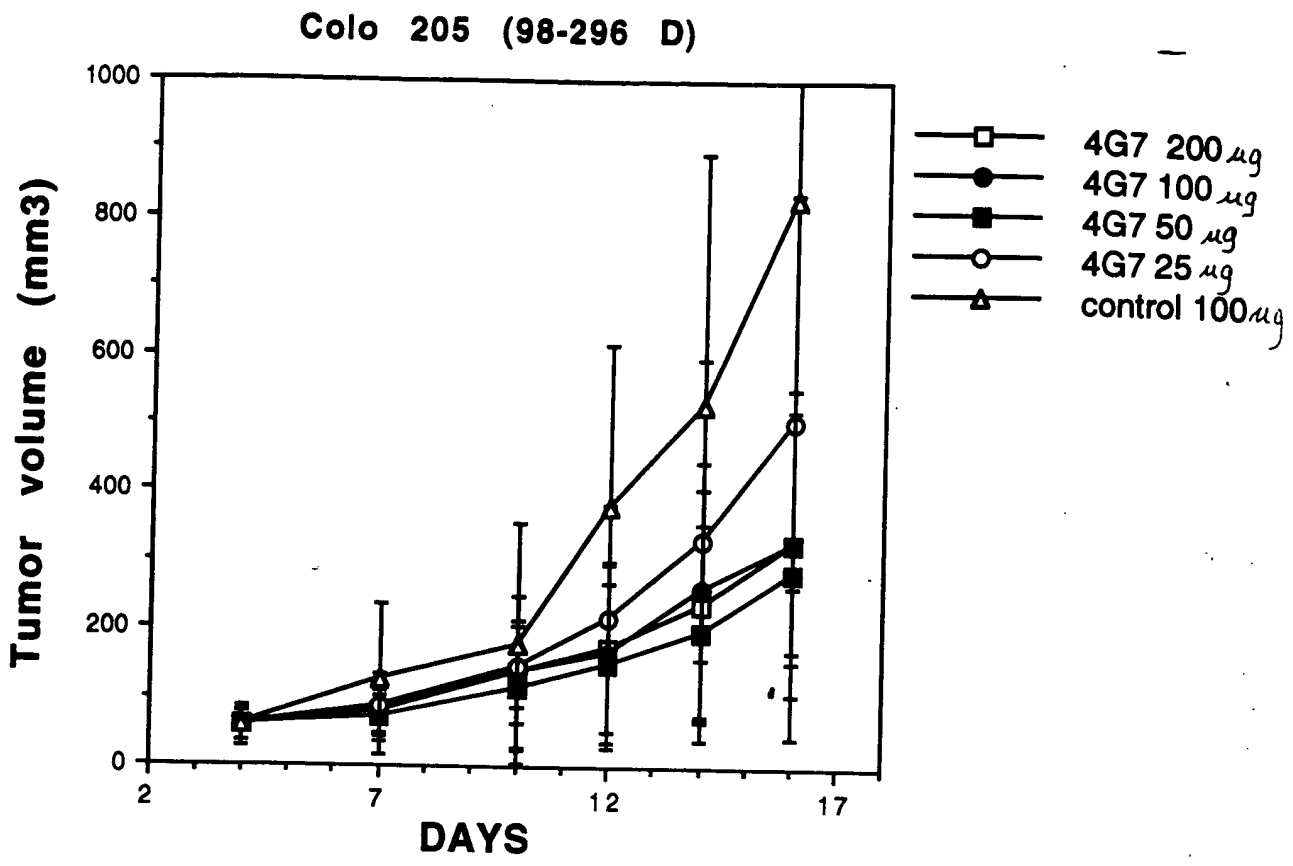

Effect of anti-DR4 mAbs
on the HCT-116 tumor Weight on Day 50
Tumor Weight (mmg)
3000
2000
1000
0
4G7
5G11
IgG2
Antibody

Fig. 14


Colo 205 (98-296 D)
Tumor volume (mm3)
1000
800
600
400
200
0
2
7
12
17
DAYS
4G7 200 ug
4G7 100 ug
4G7 50 ug
4G7 25 ug
control 100ug

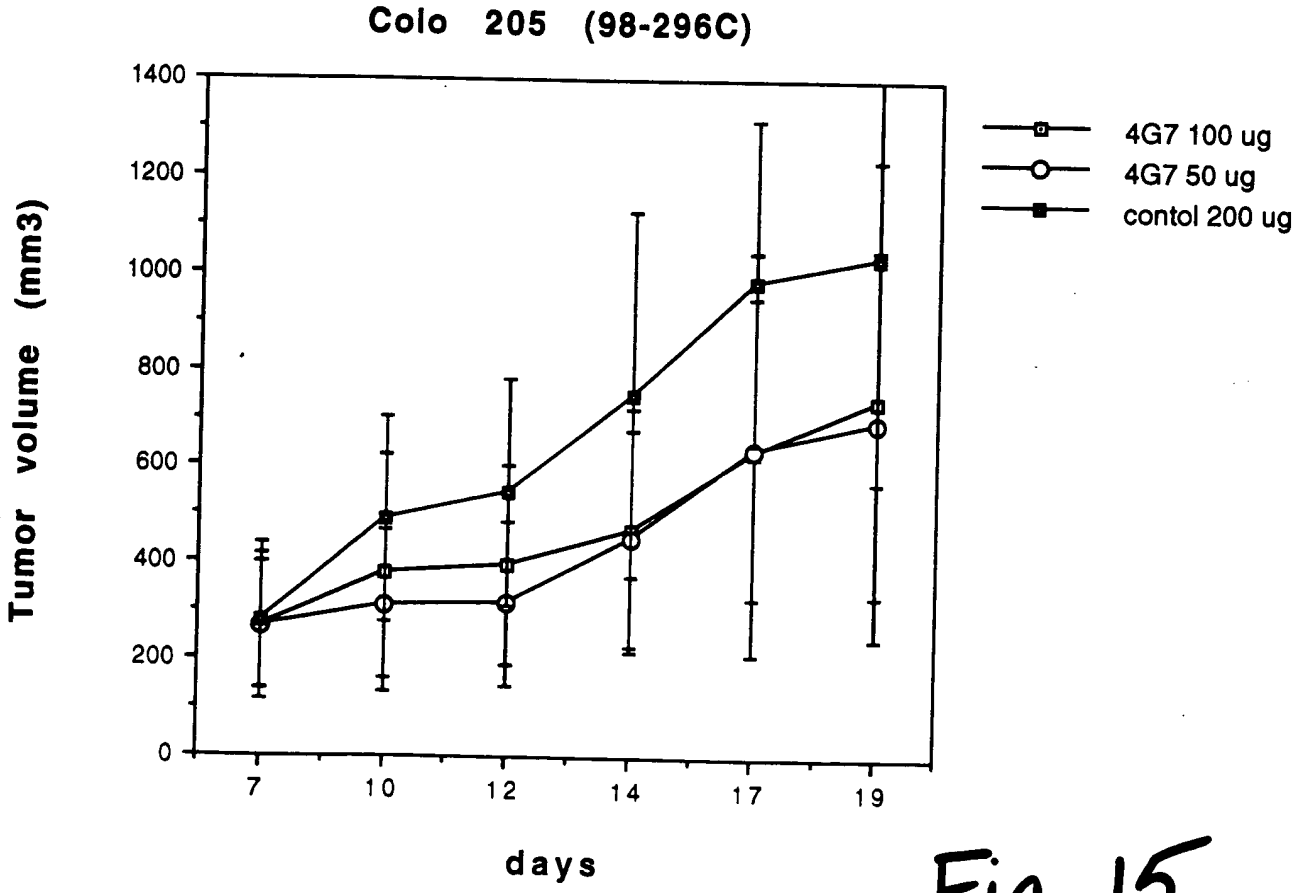

Colo 205 (98-296C)
Tumor volume (mm3)
1400
1200
1000
800
600
400
200
0
7
10
12
14
17
19
days
4G7 100 ug
4G7 50 ug
contol 200 ug

Fig. 15

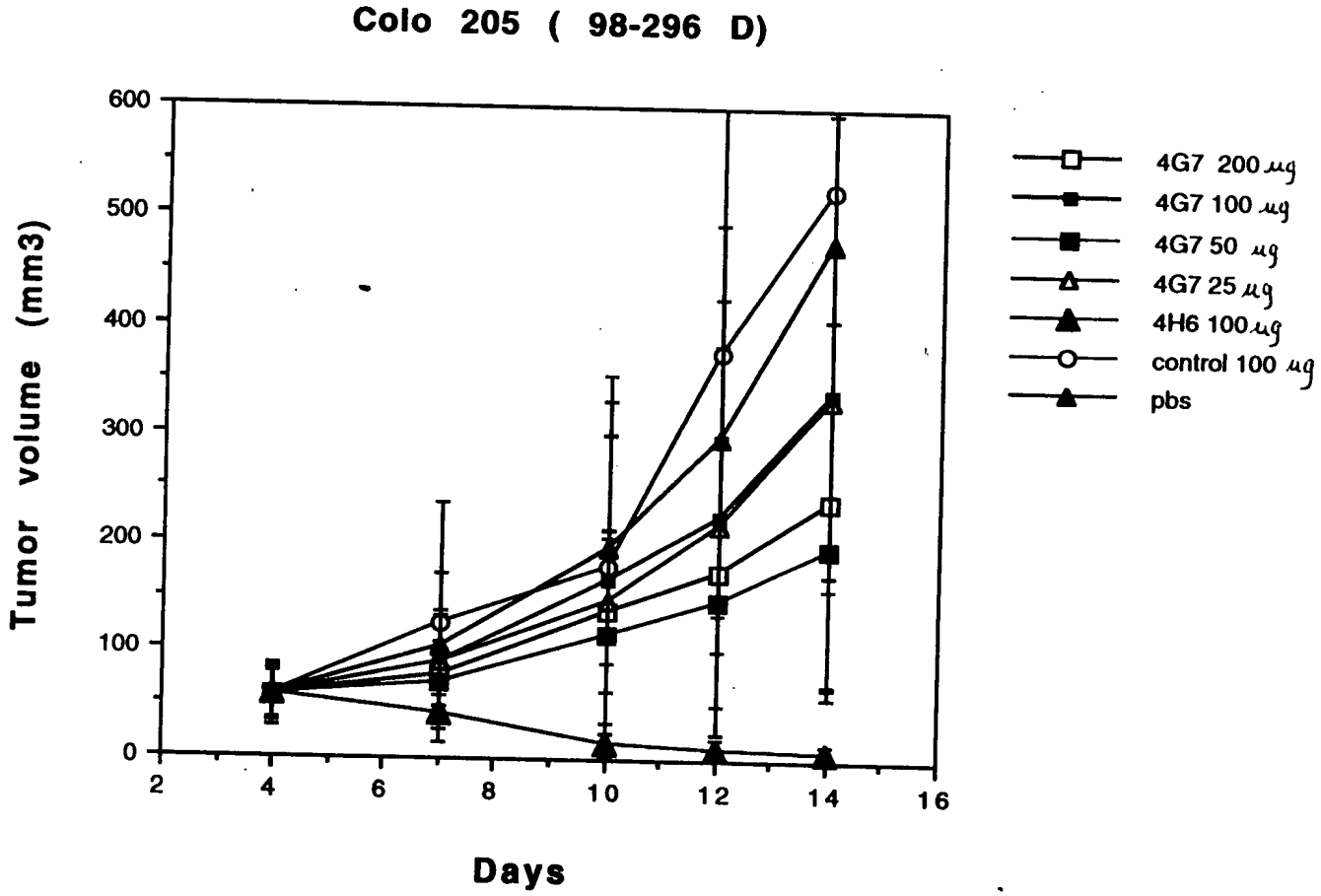

Colo 205 (98-296 D)
Tumor volume (mm3)
600
500
400
300
200
100
0
2
4
6
8
10
12
14
16
Days
4G7 200 µg
4G7 100 µg
4G7 50 µg
4G7 25 µg
4H6 100 µg
control 100 µg
pbs

Fig. 16

General Characteristics of Anti-DR4 mAbs

	Isotype	Kd-1 (pM)	Apop w/o L	Apop + &Fc	Apop + C'	Block	Cross reactivity			
							DR4	DR5	DCR1	DCR2
1H5.24.9	IgG2a			-	-	ND	+++	-	-	-
1H8.17.5	IgG1			+	ND	ND	+++	-	-	-
3G1.17.2	IgG1		-	-	ND	-	+++	-	-	-
4E7.24.3	IgG1	2	+/-	+	-	-	+++	+	-	+/-
4G7.18.8	IgG2a		+/-	+	+	-	+++	-	-	-
4H6.17.8	IgG1	5	+/-	+	-	+	+++	+	-	-
4G10.20.6	IgG1			+	ND	-	+++	+	-	-
5G11.17.1	IgG2b	22		+	+	ND	+++	++	-	-

All these mAbs recognize DR4 on 9D cells and immune precipitate DR4-IgG.
w/o L : The apoptotic ability of mAbs by themselves was detected on 9D cells, skmes cells , HCT116 and colo 205
+ &FC: The apoptotic ability of mAbs was determined in combination with goat anti-mouse IgG FC.
+ C' : The apoptotic ability of mAbs was determined in the presence of rabbit complement
Degrees of binding (+) to DR5 by Mabs 4E7 and 4H6 at 10 ug/ml are 15% of the binding to DR4.

Fig . 17